Borneo Journal of Resource Science and Technology (2023), 13(1): 165-172
DOI: https:doi.org/10.33736/bjrst.5283.2023

Morphometric Analysis of Sexual Dimorphism in *Penthicodes farinosus* (Weber, 1801) (Hemiptera: Fulgoridae) from Sarawak

AHMAD IRFAN ABDUL RAZAK*[1], SITI NURLYDIA SAZALI*[1], RATNAWATI HAZALI[1], FARAH NABILLAH ABU HASAN AIDIL FITRI[1] & ISAAC STIA MARCELLINUS[2]

[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [2]Household and Structural Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
*Corresponding author: irfanrzks@gmail.com; ssnurlydia@unimas.my
Received: 26 December 2022 Accepted: 11 June 2023 Published: 30 June 2023

ABSTRACT

The *Penthicodes farinosus* are strikingly coloured insects and can be found in India, Myanmar, Peninsular Malaysia, Java, Sumatra, Sulawesi, the Philippines, and Borneo. Despite their wide distributional range in Asia with a common occurrence in Borneo, morphometric investigation of *P. farinosus* is still lacking. The study is aimed to investigate the morphological variations of this species between two different sexes in Sarawak, Malaysian Borneo. Eleven morphometric characters were measured from 183 specimens (69 males and 114 females). The data were analysed using an independent *t*-test, Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA). Sexual dimorphism index (SDI) was found ranging from 0.044 (LV) to 0.1008 (BTg) indicating females were larger than males. In PCA, cumulative variations of 59.9% were recorded from two principal components, showing higher loadings in the length of tegmen (LTg) and total length (TL). DFA revealed a single function that explains a canonical correlation of 0.895 with 100% variation. The Wilks' Lambda values of 0.199 were highly supported with $p<0.0001$. The highest loadings for the model are LTg and TL. The two variables were further tested using Leave-One-Out Cross Validation (LOOVC) method which resulted in 97.2% cases being correctly classified as male or female. This suggests LTg and TL can be useful in separating both sexes of *P. farinosus*.

Keywords: Discriminant Function Analysis, Leave-One-Out Cross Validation, morphometric, *Penthicodes farinosus*, sexual dimorphism

INTRODUCTION

The family Fulgoridae are known for their striking colouration and spectacular ornamentation. The group has 142 genera and 774 species (Bourgoin, 2021). Ten species belonging to two subgenera being *Penthicodes* and *Ereosoma* are recognised within the genus *Penthicodes* (Nagai & Porion, 1996). Two of these species are in the subgenus *Penthicodes* namely *P. farinosus* and *P. nicobaricus* (Constant, 2010). *P. farinosus* has a distributional range in South Asia and Southeast Asian countries. The species has been recorded in India, Myanmar, Peninsular Malaysia, Java, Sumatra, Sulawesi, the Philippines, and Borneo (Nagai & Porion, 1996; Bosuang *et al*., 2017).

The species can be commonly found in Borneo (Bosuang *et al*., 2017). They feed on many different plant species from the family Fabaceae, Arecaceae, Simaroubaceae, Ebenaceae, Lamiaceae and Apocynaceae (Razak *et al*., 2020; Jiaranaisakul & Constant, 2022). *Penthicodes farinosus* has also been recorded in urban areas (Razak *et al*., 2020). The species are varied with contrasting colours, with some body parts covered in wax (Figure 1). The species are nocturnal (Natanaela *et al*., 2022) like most species in the family Fulgoridae (Naskrecki & Nishida, 2007).

Sexual dimorphism refers to a condition where two genders of a particular species exhibit differences in body size, shape, trait, colour, and parasitic load (Mori *et al*., 2017). Sexual dimorphism in *P. farinosus* is not evident unless body size is measured. Colour assessment is unreliable as this group can discolour over time. The colouration of individuals from the same gender may also vary. Observations of genitalia are the typical process to determine the sex of members from this species, which can be viewed ventrally at the apex of the abdomen.

The external male genitalia of hemipterans are composed of the aedeagus, the phallobase, the connective and the genital styles (Seidel & Weisel, 2013). On the other hand, the external female genitalia consist of pairs of plates and processes of appendicular subcoxal-coxal origin borne by abdominal segments VIII and IX (genital segments) (Bourgoin, 1993). The female genitalia are incomplete (Metcalf, 1947). Seidel and Weisel (2013) described the male genitalia of *P. farinosus* by these characters: phallus very short in its inactive state, forms bilateral symmetrical sacks and appendages when inflated in its active state; anal tube rounded dorsally, lateral margin slightly flattened running almost parallel to the entire length, paraproct bristled; genital styles rounded and paddle-shaped. Morphometrics play a vital role in the classification of Fulgoridae. For instance, the species *Pyrops cultellatus* was previously placed in the genus *Saiva*. This revision was due to the larger size and shape of the head process (Nagai and Porion, 2002). However, morphometric analysis was not conducted to support the findings of Nagai and Porion (2002). Evidently, morphometrics on Fulgoridae is greatly understudied and has only been implemented to analyse *Lycorma delicatula* (e.g. Kim, 2013; Avanesyan *et al*., 2019), an invasive planthopper species with economic importance in agriculture (Bartlett *et al*., 2018). Despite having a wide distributional range in Asia with a common occurrence in Borneo, there has been no effort to investigate the morphometrics of *P. farinosus* in this region. The objective of this study is to investigate the morphological variation of *P. farinosus* between two different sexes in Sarawak, Malaysian Borneo.

MATERIALS AND METHODS

Specimens of *Penthicodes farinosus* were examined from two institutions in Sarawak, Malaysian Borneo which includes: 1) Universiti Malaysia Sarawak Insect Reference Collection, Sarawak (UIRC); and 2) Research, Development and Innovation Division of Forest Department Sarawak, Sarawak (RDID). Species identifications were based on Nagai and Porion (1996) and Bosuang *et al*. (2017). A total of eleven morphometric characters from 183 individuals (69 males and 114 females) were measured using Mitutoyo™ digital caliper with all the measurements provided in millimetres (mm). Images of male and female *P. farinosus* were taken using Olympus Tough TG-3 (Figure 1 & 2). The morphometric characters measured include breadth of vertex (BV), length of vertex (LV), breadth of pronotum (BP), length of pronotum (LP), breadth of mesonotum (BM), length of mesonotum (LM), breadth of frons (BF), length of frons (LF), breadth of tegmen (BTg), length of tegmen (LTg) and total length (TL) following Constant (2004) (Figure 3). Drawings were made using the Procreate application on an iPad Pro based on personal observation. Specimens were examined using a Motic SMZ-168 microscope with Moticam 2000 2.0M Pixel camera and Motic Images Plus 2.0 ML software.

All eleven morphological characters were subjected to independent *t*-test, Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) using Minitab version 17.1.0 (Minitab Inc, 2010) and SPSS version 24.0 (IBM Corp, 2016) following Sazali *et al*. (2018). In each analysis, the probability of $p<0.05$ was considered significant. The sexual size dimorphism (SSD) was calculated using sexual dimorphism index (SDI) from the mean measurements between both sexes following Lovich and Gibbons (1992). The SDI was calculated using Eq. (1):

$$\mathrm{SDI} = \left(\frac{\text{mean size of female}}{\text{mean size of male}}\right) - 1 \qquad \text{Eq. (1)}$$

The values range between -1 and 1, whereby negative numbers denote males are larger than females (Lovich & Gibbons, 1992). A Leave-One-Out Cross Validation (LOOVC) method was performed through JASP 0.16.1 (JASP Team, 2022) to estimate the accuracy rate in sex discernment of *P. farinosus.*

Figure 1. Habitus image of *Penthicodes farinosus* ♀. Scale = 10 mm

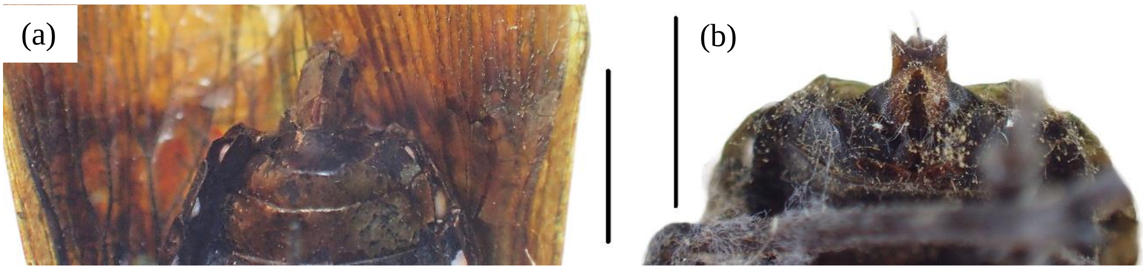

Figure 2. External genitalia of *Penthicodes farinosus* in ventral view. (a) male external genitalia; (b) female external genitalia. Scale = 5 mm

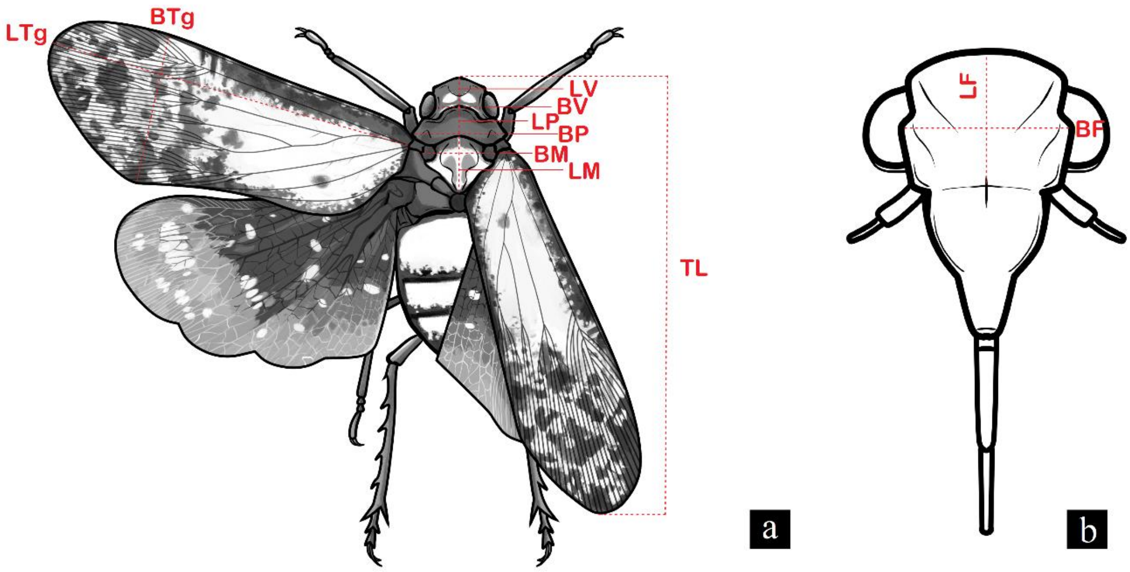

Figure 3. Morphometric characters measured which includes breadth of vertex (BV), length of vertex (LV), breadth of pronotum (BP), length of pronotum (LP), breadth of mesonotum (BM), length of mesonotum (LM), breadth of frons (BF), length of frons (LF); breadth of tegmen (BTg), length of tegmen (LTg) and total length (TL); (a) dorsal view, (b) ventral view of frons (image not to scale)

RESULTS

A total of eleven morphological characters from 183 individuals of *P. farinosus* have been measured and is summarised in Table 1. As the sample size for each species is greater than 30, the data was assumed to follow a normal distribution which satisfies the normality requirement for further statistical analysis (Sazali *et al*., 2018). Based on the independent *t*-test, all variables were found to be insignificant in differentiating the two sexes from this species except for length of frons (LF) with $p<0.05$. SDI values ranged from 0.0440 (LV) to 0.1008 (BTg) indicating females were larger than males.

Table 1. Summary of the 11 morphological characters measured (mm) in *Penthicodes farinosus* for this study

Gender	Male (n = 69)				Female (n = 114)					
Character	Mean	SD	Min	Max	*Mean*	SD	Min	Max	SDI	*p*
BF	3.4822	0.1855	3.17	3.99	3.701	0.17492	3.25	4.23	0.0628	0.99
LF	4.0370	0.34282	3.23	4.85	4.2889	0.25875	3.73	4.79	0.0624	0.002*
BV	3.2775	0.23327	2.75	3.88	3.4676	0.26658	2.77	4.19	0.0580	0.228
LV	1.8619	0.19211	1.57	2.3	1.9438	0.17799	1.54	2.37	0.0440	0.371
LP	2.2578	0.17656	1.92	2.79	2.4211	0.15946	1.87	2.77	0.0723	0.635
BP	6.8696	0.24188	6.31	7.62	7.4366	0.22401	6.84	7.98	0.0825	0.469
LM	3.6994	0.32134	3.11	4.91	3.9346	0.30531	3.2	4.91	0.0636	0.779
BM	5.9494	0.37752	4.77	6.92	6.3875	0.30766	5.62	7.17	0.0736	0.265
LTg	22.8375	0.56491	21.53	24.1	24.9919	0.57413	23.75	26.48	0.0943	0.698
BTg	8.6558	0.35465	7.79	9.44	9.5284	0.37353	8.56	10.54	0.1008	0.765
TL	27.6286	0.61988	26.1	28.8	30.1312	0.71994	28.43	31.64	0.0906	0.18

Note: Breadth of vertex (BV), length of vertex (LV), breadth of pronotum (BP), length of pronotum (LP), breadth of mesonotum (BM), length of mesonotum (LM), breadth of frons (BF), length of frons (LF), breadth of tegmen (BTg), length of tegmen (LTg) and total length (TL) and sexual dimorphism index (SDI). *Significant difference

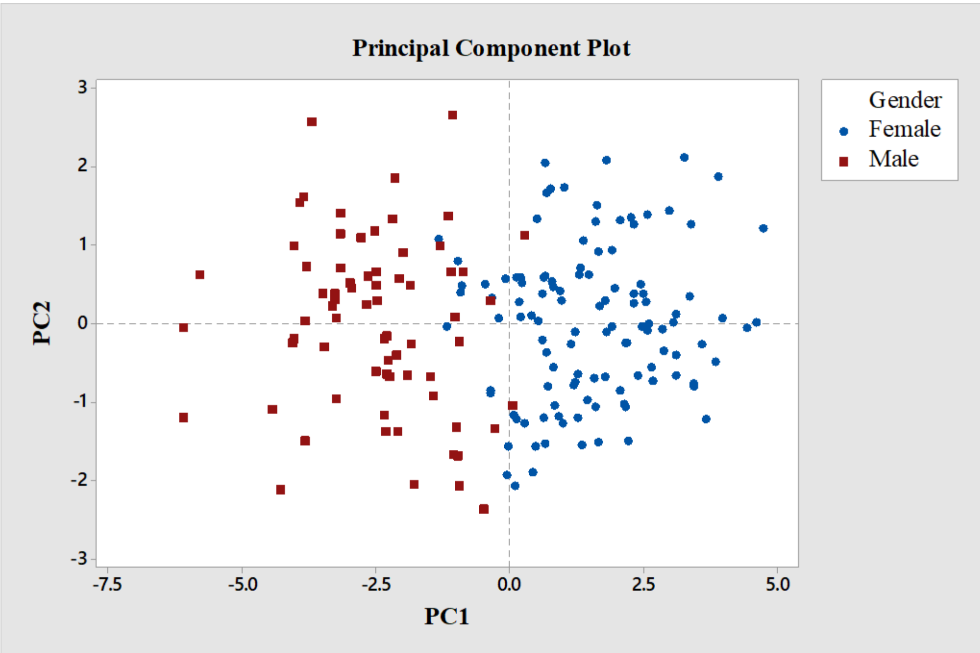

Figure 4. Principal component plot (PC1 vs PC2) between male and female of *Penthicodes farinosus*

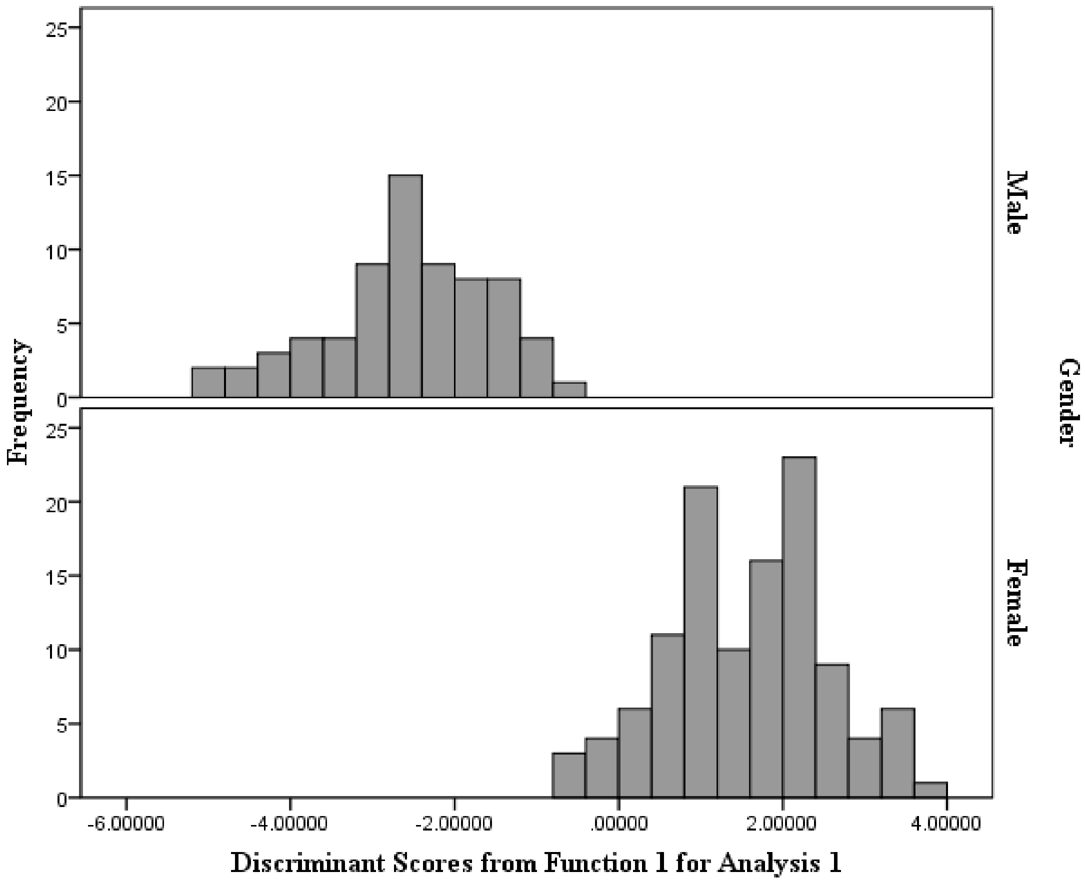

Figure 5. Separate-group histograms of discriminant function scores for male and female of *Penthicodes farinosus*

Table 2. Eigenanalysis of the Correlation Matrix

	PC1	PC2	PC3
Eigenvalue	5.5483*	1.0428*	0.9392
Proportion	0.504	0.095	0.085
Cumulative	0.504	0.599	0.685

Note: *Eigenvalue greater than 1.0.

Table 3. Principal component values of morphometric characters

Variable	PC1	PC2
Breadth of frons (BF)	0.313	-0.105
Length of Frons (LF)	0.233	-0.270
Breadth of vertex (BV)	0.238	0.213
Length of vertex (LV)	0.143	-0.511
Length of pronotum (LP)	0.221	-0.546*
Breadth of pronotum (BP)	0.381	0.056
Length of mesonotum (LM)	0.218	0.517*
Breadth of mesonotum (BM)	0.304	0.189
Length of tegmen (LTg)	0.390*	0.054
Breadth of tegmen (BTg)	0.363	0.047
Total Length (TL)	0.390*	0.022

*Diagnostic character in each function.

From the PCA (Table 2), two principal components showed the most variations between two sexes with cumulative variations of 59.9%. Following Kaiser's criterion, only an eigenvalue of 1.0 or more are retained for further analysis (Pallant, 2011). In the first principal component (PC1), characteristics that showed higher loadings were LTg (0.390) and TL (0.390) as shown in Table 3, supported with eigenvalue of 5.5483 and 50.4% variation. The second principal component (PC2) showed higher loadings at the LP (-0.546) and LM (0.517) with an eigenvalue of 1.0428 and 9.5% variation. Gender-grouping were found to be obvious forming their clusters as shown in Figure 4 with several samples overlapped.

Table 4. Eigenvalues for the DFA

Function	1
Eigenvalue	4.033[a]
% of Variance	100.0
Cumulative %	100.0
Canonical Correlation	.895

Further, DFA test revealed a single significant function that explains a canonical correlation of 0.895, accounting for 100% of the variation (Table 4). The Wilks' Lambda values of 0.199 was supported with a highly significant function of p<0.0001 (Table 5). Moreover, the standardised canonical discriminant function coefficient (Table 6) revealed the highest loadings for the model being LTg and TL. The discriminant function scores were clearly separated for both sexes with slight overlap, showing most examined parameters were useful in separating both groups (Figure 5). Two variables; LTg and TL were further tested for possible accuracy in separating the two sexes of the species. A total of 147 training and validation datasets, and 36 test datasets were used in the analysis. Through the LOOVC method, 97.2% of cases were correctly classified (Table 7). The algorithm correctly predicts 11 males and 24 females resulting in a test accuracy of 35/36 = 0.972 (Table 8). Thus, this strongly suggests that LTg and TL can be useful in identifying the sex of *P. farinosus* accurately.

Table 5. Wilks' Lambda values for the DFA

Test of Function(s)	1
Wilks' Lambda	.199
Chi-square	283.608
df	11
Sig.	.000

Table 6. Standardised canonical discriminant function coefficients for the 11 characters of *Penthicodes farinosus*

Variable	Function 1
Breadth of frons (BF)	-.186
Length of Frons (LF)	.044
Breadth of vertex (BV)	-.108
Length of vertex (LV)	-.005
Length of pronotum (LP)	.103
Breadth of pronotum (BP)	.206
Length of mesonotum (LM)	-.026
Breadth of mesonotum (BM)	-.138
Length of tegmen (LTg)	.534*
Breadth of tegmen (BTg)	.173
Total Length (TL)	.426*

Note: *Diagnostic character(s) with high coefficient in Function 1.

Table 7. Accuracy result from Leave-One-Out Cross Validation (LOOVC) test in gender classification of *Penthicodes farinosus* using morphometric data

K-Nearest Neighbors Classification	
Nearest neighbors	1
Weights	rectangular
Distance	Euclidean
n(Train)	146
n(Validation)	1
n(Test)	36
Validation Accuracy	1.000
Test Accuracy	0.972

Note: The model is optimised with respect to the validation set accuracy.

Table 8. Summary of prediction results from confusion matrix on the gender classification of *Penthicodes farinosus*

		Predicted	
		Male	Female
Observed	Male	11	1
	Female	0	24

DISCUSSION

Little is known on the measurements of *Penthicodes farinosus*, except for wingspan of males (46 − 54 mm) and females (55 − 59 mm) as reported by Bosuang *et al*. (2017). However, Bosuang *et al*. (2017) did not provide the number of individuals measured. In the current study, females of *P. farinosus* were found larger than males with SDI ranging from 0.0440 (LV) to 0.1008 (BTg). The females of Fulgoridae tends to be larger than the opposite sex (Nagai & Porion, 2002; Bosuang *et al*., 2017), but this is not universal to all species from the genus *Penthicodes*. The females from the species *P. bimaculatus* were reported to be smaller than the males in total length (Constant, 2010). Female-biased SSD predominantly occurs in most insect species as females develop through a higher number of instars than males (Mori *et al*., 2017). Also, scramble competition for access to mates with direct and indirect female choice is linked with female-biased SSD (Blanckenhorn, 2005).

In both PCA and DFA, the differences between both sexes were found in LTg and TL. Insect wings and thorax are usually under stabilising natural selection unless involved in courtship (Gilchrist *et al*., 2000; Rohner & Blanckenhorn, 2018). The wing length of

Diptera is greater for highly mobile females (Mori *et al*, 2017). In Fulgoridae, wing area has a negative correlation with flight ability as the heaviest females of *Lycorma delicatula* had larger wings but flew poorly, with the lightest female having small wings (Wolfin *et al*., 2019). As such, the weight of an individual dictates the size of wing. Body and tissue size in animals are determined by genetic and environmental factors, but the exact mechanism by which tissue size is proportioned to body size are unknown (Dhungel & Otaki, 2014). Despite their lack of involvement in copulation, the study revealed that LTg and TL could be used for the sex discernment of *P. farinosus*.

Currently, relying solely on LTg and TL for discerning the sex of *P. farinosus* seems premature as several individuals from both sexes share similar measurements used in the current study. Other morphological characters should be considered to see their viability in identifying the sex of *P. farinosus*. For example, mouthparts (stylets and labium) and tarsal tips (arolia and tarsal claws) were measured by Avanesyan *et al*. (2019) when assessing changes in the morphology of *Lycorma delicatula* at each developmental stage. Breadth of head process and length of head process should be measured for species with a head process. Increasing sample size could also improve the estimation to be more precise. Using morphometrics as a diagnostic indicator in sex identification could reduce the time consumption necessary for morphological observations as well as limit the need for manual handling of specimens.

CONCLUSION

Two morphological characters were found significant in identifying the sex of *P. farinosus* namely length of tegmen and total length. Further sampling to increase sample size could enhance the accuracy of gender-grouping for this species. As the biology, life history, behaviour, and phenology of Fulgoridae remains undocumented, future morphometric studies on Fulgoridae should be considered as this group of insects is greatly understudied.

ACKNOWLEDGEMENTS

This study was partially funded under the UNIMAS Special Top-Down Grant (F07/SpTDG/1766/ 2018). The authors wish to thank the UNIMAS Insect Reference Collection (UIRC) and the Research Development and Innovation Division, Forest Department Sarawak (RDID) for providing facilities and granting permission to examine the voucher specimens kept in the insect repository.

REFERENCES

Avanesyan, A., Maugel, T.K. & Lamp, W.O. (2019). External morphology and developmental changes of tarsal tips and mouthparts of the invasive spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). *PloS One*, 14(12): e0226995.

Bartlett, C.R., Deitz, L.L., Dmitriev, D.A., Sanborn, A.F., Soulier-Perkins, A. & Wallace, M.S. (2018). The diversity of the true hoppers (Hemiptera: Auchenorrhyncha). *Insect Biodiversity: Science and Society*, 2: 501-590.

Blanckenhorn, W.U. (2005). Behavioral causes and consequences of sexual size dimorphism. *Ethology*, 111(11): 977-1016.

Bosuang, S., Audibert, C., Porion, T. & Chan, C.L. (2017). A guide to lanternflies of Borneo. Malaysia: Natural History Publications (Borneo). pp. 5-110.

Bourgoin, T. (1993). Female genitalia in Hemiptera Fulgoromorpha, morphological and phylogenetic data. *Annales de la Société Entomologique de France (N.S.)*, 29(3): 225-244.

Bourgoin, T. (2021). FLOW (Fulgoromorpha Lists on The Web): A World Knowledge Base Dedicated to Fulgoromorpha Version 8, Retrieved February 3, 2022 from http://hemiptera-databases.org/flow/.

Constant, J. (2004). Révision des Eurybrachidae (I). Le genre Amychodes Karsch, 1895 (Homoptera: Fulgoromorpha: Eurybrachidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique*, 74: 11-28.

Constant, J. (2010). The lanternfly genus *Penthicodes*: Key to the species and review of the "Ereosoma group" with two new species and one new subspecies (Hemiptera: Fulgoromorpha: Fulgoridae). *Zootaxa*, 2523: 1-26.

Dhungel, B. & Otaki, J.M. (2014). Morphometric analysis of nymphalid butterfly wings: number, size and arrangement of scales, and their implications for tissue-size determination. *Entomological Science*, 17(2): 207-218.

IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Gilchrist, A.S., Azevedo, R.B.R., Partridge, L. & O'higgins, P. (2000). Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. *Evolution & Development*, 2(2): 114-124.

JASP Team (2022). JASP (Version 0.16.1)[Computer software].

Jiaranaisakul, K. & Constant, J. (2022). New records of *Penthicodes* lanternfly species from Thailand and Malaysia and nomenclatural notes on the genus (Hemiptera: Fulgoromorpha: Fulgoridae). *Bonn Zoological Bulletin*, 71(2): 87-97.

Kim, H. (2013). Morphometric analysis of wing variation of lantern fly, *Lycorma delicatula* from northeast Asia. *Korean Journal of Applied Entomology*, 52 (4): 265-271.

Lovich, J.E. & Gibbons, J.W. (1992). Review of techniques for quantifying sexual size dimorphism. *Growth, Development and Aging*, 56(4): 269-81.

Metcalf, Z.P. (1947). *Fascicle IV, Fulgoroidea. part. 9, Fulgoridae. General catalogue of the Hemiptera*. Northhampton, Massachusetts: Smith College.

Minitab 17 Statistical Software (2010). [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com).

Mori, E., Mazza, G. & Lovari, S. (2017). Sexual dimorphism. In Vonk, J. & Shakelford, T. (Eds.). *Encyclopedia of animal cognition and behavior*. Springer International Publishing: Switzerland. pp. 1-7.

Nagai, S. & Porion, T. (1996). *Fulgoridae 2: Illustrated catalogue of the Asiatic and Australianfauna*. France: Sciences Nat, Venette. Pp. 1-80.

Nagai, S. & Porion, T. (2002). *Fulgoridae 2: Supplement 1: New Fulgoridae from South-East Asia*. Canterbury: Hillside Book. pp. 1-15.

Naskrecki, P. & Nishida, K. (2007). Novel trophobiotic interactions in lantern bugs (Insecta: Auchenorrhyncha: Fulgoridae). *Journal of Natural History*, 41(37-40): 2397-2402.

Natanaela, J., Putra, G.Y., Runtulalo, S. & Luntungan, E.M. (2022). Inventarisasi komunitas Arthropoda di Taman Kehati Kaki Dian, Minahasa Utara. *Silvarum*, 1(3): 75-81.

Pallant, J. (2011). *SPSS survival manual: A step by step guide to data analysis using IBM SPSS*. Australia: Allen and Unwin. pp. 1-336.

Razak, A.I.A., Sazali, S.N., Hazali, R. & Fitri, F.N.A.H.A. (2020). Plant association of lanternflies (Hemiptera: Fulgoridae) from Malaysian Borneo. *Borneo Journal of Resource Science and Technology*, 10(2): 172-178.

Rohner, P.T. & Blanckenhorn, W.U. (2018). A comparative study of the role of sex-specific condition dependence in the evolution of sexually dimorphic traits. *The American Naturalist*, 192 (6): E202-E215.

Sazali, S.N., Hazmi, I.R., Abang, F., Rahim, F. & Jemain, A.A. (2018). Morphometric study of the palm weevils, *Rhynchophorus vulneratus* and *R. ferrugineus* (Coleoptera: Curculionidae) in view of insular and mainland populations of Malaysia. *Pertanika Journal of Tropical Agricultural Science*, 41(3): 1329-1340.

Seidel, S. & Wessel, A. (2013). On the morphology and preparation of male genitalia in the Fulgoridae – the Southeast Asian genus *Penthicodes* (Hemiptera, Fulgoromorpha, Fulgoridae). *Deutsche Entomologische Zeitschrift*, 60(2): 193-207.

Wolfin, M.S., Binyameen, M., Wang, Y., Urban, J.M., Roberts, D.C. & Baker, T.C. (2019). Flight dispersal capabilities of female spotted lanternflies (*Lycorma delicatula*) related to size and mating status. *Journal of Insect Behavior*, 32 (3): 188-200.